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(54) Title: TREATMENT OF AUTOIMMUNE DISEASES

(57) Abstract

A method for isolating a TABM capable of suppressing an autoimmune response characteristic of a human autoimmune disease, said method comprising culturing T-cells which have been exposed to an antigen to which patients with said disease produce an autoimmune response, to stimulate TABM production by said T-cells, isolating from said culture cells or their supernatant material capable of binding to said antigen to produce an antigen-binding fraction, and isolating from said antigen-binding fraction a sub-fraction capable of delaying the onset or alleviating symptoms of said autoimmune disease.

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TREATMENT OF AUTOIMMUNE DISEASES

Background of the Invention

This invention relates to the treatment of autoimmune diseases.

An autoimmune disease is characterized by an immune response directed against constituents of the patient's own bodily tissues, causing damage to those tissues. One such disease is systemic lupus erythematosis (SLE), in which the patient's immune system manufactures a wide variety of autoantibodies against nuclear and cytoplasmic components of the patient's own cells.

Rheumatoid arthritis (RA), juvenile rheumatoid arthritis (JRA), psoriatic arthritis (PSA), autoimmune uveitis (AUV), polychondritis (PC) and steroid responsive hearing loss (SRHL) are serious diseases thought to have an autoimmune pathogenesis. RA, JRA and PSA are types of inflammatory arthritis. Chronic inflammatory arthritis, which results in a degree of permanent crippling in about 20-30% of those afflicted, is termed RA in adults and JRA in children; when it occurs in concert with inflammatory skin disease, psoriasis, it is termed PSA. RA afflicts approximately 1-3% of the population worldwide. JRA is the most prevalent rheumatic disease of childhood, thought to affect 66 per 100,000 with an annual incidence of 9 per 100,000 (Petty (1979) in Juvenile Rheumatoid Arthritis (Miller, ed.), PSG Pub. Co. Littleton, MA, pages 11-31). RA, JRA and PSA together affect an estimated 6-8 million people in the United States. AUV may occur in conjunction with JRA or, less frequently, independent of arthritis in both children and adults. Between 5 and

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47% of patients with JRA-associated uveitis develop blindness in at least one eye (Rosenberg, Semin. Arthritis Rheum. 16:158-173, 1987). PC and SRHL are relatively uncommon but potentially serious diseases that involve the ear (PC and SRHL), nose, trachea, larynx and other cartilagenous structures (PC).

Although the pathogenesis of RA, JRA, PSA, AUV, PC and SRHL is unknown, autoimmunity to type II collagen (CII) has been implicated. CII is a structural protein present in the hyaline cartilage found in joints, including those in the inner ear, and in the ocular vitreous (Bornstein (1980) An. Rev. Biochem. 49:957-1003). Animal analogs of autoimmune inflammatory arthritis, uveitis, and hearing loss can be experimentally induced by immunization with native CII (Breedveld et al. (1987) Rheum. Dis. Clin. N. Am. 13:531-544). When immunologically susceptible rodents (Trentham et al. (1977) J. Exp. Med. 146:857-868; Courtney et al. (1980) Nature 283:666-668) or primates (Cathcart et al. (1986) Lab. Invest. 54:26-31) are immunized with native, but not denatured, CII, there is induced an acute and sustained polyarthritis resembling rheumatoid arthritis, a chondritis similar to polychondritis (Cremer et al. (1981) J. Exp. Med. 154:535-540; McCune et al. (1982) Arthritis and Rheum. 25:266-273), or an anterior uveitis associated with arthritis mimicking juvenile rheumatoid arthritis (Trentham et al. (1989) Invest. Ophthalmol. Vis. Sci. 30:228; O'Brien et al. (1989) Arthritis Rheum. 32:S89). In humans, T-cell response to type II collagen can be demonstrated in vitro in 80-100% of patients with RA, JRA, PSA, and PC, even early in the course of their disease. (Trentham et al. (1978) N. Engl. J. Med. 299:327-332; Rosenberg et

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al. (1984) J. Rheum. 11:425-431; Trentham et al. (1981) Arthritis Rheum. 24:1363-1369; Foldart et al. (1978) N. Engl. J. Med. 299: 1203-1207. In contrast, patients with other forms of arthritis and eye disease do not have T-cells which are responsive to type II collagen.

T-cells appear to play a fundamental role in the autoimmune response in all these diseases. In inflammatory arthritis, for example, T-cell-specific antiserum given to rats confers immunity in animal models of inflammatory arthritis (Breedveld et al. (1987)). In addition, collagen arthritis can be passively transferred in rats by injection of as few as 10^3 collagen reactive T cells into the knee joint of syngeneic, immunologically naive rats (Brahn et al. (1989) Cell. Immunol. 118:491-503).

The mechanism by which T cells may participate in inflammatory arthritis may be explained by a protein known as arthritogenic factor (AF). AF is a 65Kd, collagen binding T-cell antigen binding molecule (TABM) (Helfgott et al. (1985) J. Exp. Med. 162:1531-1545; Helfgott et al. (1988) J. Immunol. 140:1838-1843; Caulfield et al. (1988) Lab. Invest. 59:82-95). The primary function of TABMs appears to be immunoregulatory. Hapten- or antigen specific TABMs have been described in the mouse with T helper, T suppressor-inducer, and even T contrasuppressor capabilities (Cone et al. (1987) Method. Enzymol. 150:666-681; Cone et al. (1988) Intern. Rev. Immunol. 3:205-228).

Summary of the Invention

The invention provides a method for the isolation of TABM's capable of suppressing a specific autoimmune response and for the use of these TABM's in

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treating autoimmune diseases. Autoimmune diseases are characterized by autoimmunity against components of affected tissue: in SLE, e.g., the patient has autoantibodies against a variety of cellular components including DNA and RNA. In the collagen-autoimmunity diseases such as RA, the patient has autoimmunity to CII, and in multiple sclerosis (MS) the patient exhibits autoimmunity to myelin basic protein.

The invention provides for the isolation of TABM's that specifically suppress an autoimmune response of a given autoimmune disease, e.g., in SLE an anti-DNA response, in collagen autoimmune diseases, the anti-collagen response, and in MS, the anti-myelin basic protein response. The TABM's can be expected to have acceptably low toxicity since the action of each will specifically suppress an immune response to a particular antigen and would not be expected to cause a general or broad spectrum suppression of the immune system.

TABM's capable of suppressing an autoimmune response characteristic of a human autoimmune disease are isolated by a method that includes the following steps: (1) culturing T-cells which have been exposed to an antigen to which patients with the disease produce an autoimmune response, to stimulate TABM production by the T-cells; (2) isolating from the cultured cells, or their supernatant, material capable of binding to the antigen to produce an antigen-binding fraction; (3) isolating from said antigen-binding fraction a sub-fraction capable of delaying or alleviating symptoms of the autoimmune disease.

In preferred embodiments the T-cells are obtained from a human patient suffering from the

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autoimmune disease. In other preferred embodiments to the T-cells are obtained from a non-human mammal which has been immunized with the antigen. The T-cells may be cultured in the presence of the antigen.

The invention features therapy useful in treating, preventing, or delaying the onset of diseases caused by collagen-autoimmunity using a substantially pure protein called anti-arthritis factor (AAF). AAF has the following characteristics: (a) it has a mass of approximately 55 kd as determined by SDS-PAGE; (b) it is found in, or on the surface of, collagen stimulated T-cell antigen binding molecule secreting cells; (c) it is capable of binding collagen; (d) it reduces the incidence and severity and delays the onset of arthritis in rats immunized with collagen; and (e) and it increases the titer of anti-collagen antibodies when injected into collagen immunized rats. Substantially pure means a preparation with a purity of 90% or greater by weight including no more than 10% by weight of the proteins, lipids, and carbohydrates with which the protein is naturally associated. In preferred embodiments AAF has the amino acid composition ASX 9.3; GLX 11.5; SER 13.8; GLY 10.4; HIS 1.3; ARG 3.1; THR 6.2; ALA 7.7; PRO 5.9; TYR 3.2; VAL 7.7; MET 2.9; ILE 3.4; LEU 6.6; PHE 3.0; LYS 3.9. In preferred embodiments the N-terminal amino acid sequence of AAF is ASP-SER-LEU-THR-GLN-PRO-SER.

AAF of the invention may be prepared by any method, including conventional methods of protein purification or by recombinant DNA and genetic engineering technology. The invention includes a DNA sequence encoding AAF, expression vectors containing AAF encoding DNA, and cells transformed with vectors containing DNA encoding AAF. Fragments of AAF, produced

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by methods well known to those in the art, e.g., by proteolytic degradation or by genetic engineering methods, that possess the ability to reduce the incidence, severity, or delay the onset of arthritis in rats are also useful as molecules of the invention.

Animals, preferably humans, suffering from a collagen-autoimmune disease can be treated by administration of AAF, in amounts sufficient to decrease the symptoms of the collagen-autoimmune disease. Collagen autoimmune diseases are diseases which exhibit autoimmunity to collagen and include inflammatory arthritis, e.g., RA, JRA, and PSA, AUV, PC, and SRHL.

Some of the methods and molecules of the invention allow treatment of CII-autoimmune diseases that is potent, rapid in onset, has low toxicity, and that selectively suppresses collagen-autoimmunity without interfering with the normal function of the immune system. The efficacy of this treatment relies on the presence of collagen-autoimmunity in the patients and the ability of AAF to act as a T suppressor-inducer factor to suppress the aberrant anti-self, i.e., anti-collagen, response.

An autoimmune disease, as used herein, means a disease wherein an immune response is directed against constituents of the patient's own tissues, causing damages to those tissues. An autoimmune response characteristic of an autoimmune disease is an immune response directed against a component of a tissue affected by its autoimmune disease.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiment, and from the claims.

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Description of the Preferred Embodiment

The drawings are first briefly described.

Drawings

Fig. 1 is a graph illustrating the effect of AAF on the prevalence of arthritis in collagen immunized animals.

Fig. 2 is a graph illustrating the effect of AAF on the day of onset of arthritis in collagen immunized animals.

Fig. 3 is a graph illustrating the effect of AAF on anti-collagen antibody titer in collagen immunized animals.

Preparation of AAF

AAF was isolated by affinity chromatography from the supernatent of cultured antigen-reactive T cells, as described in detail below.

The following animal strains were used in this work (any strain listed can be used both to produce AAF and as an experimental animal in the bioassay described below): Louvain (LOU) of either sex, originally derived from breeding pairs (Small Animal Facility, National Institutes of Health, Bethesda, MD), and maintained as a colony at the Animal Research Center of Harvard Medical School (Boston, MA); Female Lewis (LEW) (Marlan Sprague Dawley, Indianapolis, IN); Wistar-Furth (W-F) (Harlan Sprague Dawley, Indianapolis, IN); or male DBA/1 and DBA/2 mice (The Jackson Laboratory, Bar Harbor, ME). Histocompatibility in these strains was verified by mixed lymphocyte culture techniques (Helfgott et al. (1985) J. Exp. Med. 162:1531-1545). Random serum samples did not contain antibodies to mycoplasma, Sendai virus, sialodacryoadenitis virus, mouse adenovirus, rheovirus (strain 3), pneumonia virus of mice, Kilham's

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rat virus, or hamster osteolytic virus (Tufts University School of Veterinary Medicine, Medford, MA). Rats weighed 100-125 grams and mice weighed 25-30 grams.

To increase AAF production, rats used to produce AAF were fed minocycline hydrochloride (Lederle Pharmaceuticals, Pearl River, NJ) from the day of immunization (day 0) until the time of sacrifice. Dosing was accomplished by adding minocycline to their drinking water at 0.8 mg/ml. AAF production is greatly increased by aminocycline administration.

Antigen-reactive T cell lines were established as follows. T cell lines were established as previously described (Helfgott et al. (1985) J. Exp. Med. 162:1531-1545). Lymph node cells (LNC) and/or spleen cells were obtained from rats or mice 7 to 14 days after injection with CII (as described below) and suspended at a density of 5×10^6 cells/ml in Dulbecco's modified Eagle's medium (DMEM) (Microbiological Associates), supplemented with 1% fresh rat serum, 2-mercaptoethanol (2-ME) (5×10^{-5} M), penicillin-streptomycin, and HEPES (GIBCO, Grand Island, NY) (culture medium). LNC were cultured in 100-mm petri dishes (6 ml/dish) with the antigen CII (Helfgott et al. (1985) J. Exp. Med. 162:1531-1545). After 72 h of incubation, the cells are harvested and the lymphoblasts separated by centrifugation with a discontinuous Ficoll gradient (Helfgott et al. (1985) J. Exp. Med. 162:1531-1545). A fraction containing > 90% lymphoblasts was consistently found in the interface between densities of 1.06 and 1.05 g/ml. These cells were recovered by pipetting, washed twice, and cultured at a density of 2×10^5 cells/ml in DMEM supplemented with 20% (v/v) supernatant containing interleukin-2 (IL-2, 50

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units/ml) (described below), 10% horse serum (GIBCO), 2-ME, nonessential amino acids, sodium pyruvate, antibiotics, and HEPES, without added antigen (propagation culture medium). The IL-2 containing supernatant was prepared with Concanavalin-A stimulated BDA/2 splenocytes (Helfgott et al. (1985) J. Exp. Med. 162:1531-1545), or was derived from commercially purchased rat polyclone or recombinant IL-2 (Collaborative Research, Cambridge, MA). The isolated lymphoblasts that are propagated represent approximately 1% of the starting LNC population.

After 7 days, cells undergoing further selection were harvested and resuspended. 2×10^5 cells/ml together with 10^7 cells/ml irradiated (1500 R) syngeneic accessory cells (from the spleen of naive rats or mice) and CII antigen (10-20 μ g/ml) were suspended in culture medium (described above). Native type II collagen, prepared by pepsin treatment of lathyritic chick sternal cartilage by a method previously described (Helfgott et al. (1985) J. Exp. Med. 162:1531-1545), was obtained commercially (Genzyme Corp., Boston, MA) or was purified in our laboratory. Collagens were tested for purity by amino acid analysis and disc SDS-PAGE (Helfgott et al. (1985) J. Exp. Med. 162:1531-1545).

After 72 h of incubation, the cells were collected, washed, and applied to a Ficoll-Hypaque gradient to isolate viable lymphoblasts. The lymphoblasts were resuspended in propagation culture medium (2×10^5 cells/ml) and reseeded (10 ml/dish). After 3 to 4 days of incubation, the cells were harvested and restimulated with antigen, as described above. Supernatants from which AAF is isolated are collected at the end of an antigen-stimulation cycle. Rat T-cell membrane markers are identified by flow cytometry using murine monoclonal

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antibodies W3/13, W3/25, and Ox8 (Accurate Chemical and Scientific Corp., Westbury, NY) and fluorescein-conjugated F(ab')₂-fragments of goat anti-mouse IgG (Fc fragment-specific) (Cappel Laboratories, Cochranville, PA).

AAF was isolated from cell supernatants by affinity chromatography as follows. Supernatant material, derived from serum-containing cultures of line cells, were depleted of albumin by using Blue Sepharose chromatography (Helfgott et al. (1985) J. Exp. Med. 162:1531-1545). Forty milligrams of lyophilized crude supernatant material were solubilized in 0.05 M Tris-HCl with 0.1 M KCl (pH 7.0) and applied to a preequilibrated 0.9 x 30 cm column containing BS CL-6B (Pharmacia Fine Chemicals, Piscataway, NJ). Fractions of unbound material with an OD at 280 nm (OD₂₈₀) of > 0.05 are pooled (BS peak I). Bound material, including albumin, is eluted with 0.05 M Tris-HCl and 0.2 M NaSCN, pH 8.0 (BS peak II). Both pools were vacuum dialyzed to approximately 1/20 their original volume. Depletion efficiency was assessed by radial immunodiffusion, using 1.5% agarose containing a 1/50 dilution of rabbit anti-horse albumin (Cappel Laboratories). Up to 96% of the albumin was removed from BS peak I by this procedure.

For the final affinity isolation, CII was linked to CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals) (Helfgott et al. (1985) J. Exp. Med. 162:1531-1545). CII was prepared as described above. After the ligand was bound and the gel reequilibrated with the coupling buffer (0.01 M PBS with 0.5 M NaCl), 4 ml of BS peak I in phosphate buffered saline (PBS) was added to the gel and incubated overnight at 4° C with rocking. A 1 x 6 cm column was filled with the gel and

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washed with PBS until the OD₂₈₀ was < 0.01 and the wash discarded. A 0.2 M glycine-HCl wash, pH 2.8, was pumped onto the column and the eluate collected until the OD₂₈₀ was equal to 0.00. Elution profiles were monitored at OD₂₈₀ and concentrations determined by the BioRad protein assay (BioRad Laboratories, Richmond, CA) using BSA as a standard (Helfgott et al. (1985) J. Exp. Med. 162:1531-1545). The eluate was dialyzed for 48 h against distilled water, lyophilized, and stored at -20° C. Batch production of AAF is easily accomplished by a scale-up of this technique. Because AAF also binds to types IX and XI collagens, these proteins, or renatured CNBr fragments of types II, IX, or XI collagens, can also be used for affinity isolation although with a loss of efficiency.

The 55kd AAF species was separated from the 65kd AF species by SDS-PAGE. The AAF containing band is cut from the gel and AAF eluted, free of AF, from the excised band. Discontinuous SDS-PAGE was performed as described in Helfgott et al. (1985) J. Exp. Med. 162:1531-1545. All samples were run under reduced conditions using 2-ME in ten percent polyacrylamide, 1.5-mm-thick slab gels. Gels were stained with silver nitrate, which detects quantities of protein > 10 ng. Relative molecular weight was determined using the following BioRad protein standards: phosphorylase b (92,500), BSA (66,200), OVA (50,000 and 45,000), carbonic anhydrase (31,000), and soybean trypsin inhibitor (21,500).

Characterization of AAF

The 55 kd AAF species was routinely identified by SDS-PAGE, as described above, after affinity purification and can be easily separated from the 65 kd

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species, AF, by excising the AAF band from the gel and eluting the AAF.

If required, the species can be further verified by Western blot, N-terminus sequence analysis (ASP-SER-LEU-THR-GLN-PRO-SER), or amino acid analysis (ASX 9.3; GLX 11.5; SER 13.8; GLY 10.4; HIS 1.3; ARG 3.1; THR 6.2; ALA 7.7; PRO 5.9; TYR 3.2; VAL 7.7; MET 2.9; ILE 3.4; LEU 6.6; PHE 3.0; LYS 3.9).

AAF is an anti-Arthritic Agent

Bioactivity was confirmed by in vivo assay performed as follows. On day 0, 20 Sprague-Dawley rats were immunized with collagen, as described below. CII was prepared as described above. On day -3 and day 0 (immediately prior to immunization with CII), 10 of the rats were injected intravenously with 1-10 µg of affinity purified material containing AAF. The incidence, severity, and day of onset of arthritis was compared to the 10 collagen immunized but uninjected control rats. A significant suppression of collagen arthritis was seen in the group receiving AAF. Fig. 1 shows that the prevalence of arthritis was significantly ($p < 0.03$) suppressed. In Fig. 1 open boxes represent control and filled diamonds represent AAF injected animals. Percent arthritis was determined as described in Trentham et al. (1977) 146: 857-866. Fig. 2 shows that the onset of arthritis was delayed significantly ($p \leq 0.0005$) in AAF treated rats. Solid bars indicate control and crosshatched bars indicate AAF treated animals. The mean day of onset for experimental animals was 12.3 ± 0.4 and 11.0 ± 0.3 for control animals. The experiment was scored as in Trentham et al. (1977) 146: 857-866. Potency was also assayed by the ability of AAF to increase the anti-CII

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titer. Sera was obtained 20 days after immunization with CII and IgG antibody titers to CII measured by ELISA (Helfgott et al. (1985) J. Exp. Med. 162: 1531-1545). Fig. 3 shows a significant ($p \leq 0.01$) increase of anti-CII antibodies in the AAF treated animals. In Fig. 3 the solid bar represents the mean for control animals and the cross-hatched bar represents the mean for AAF treated animals. The experiment was scored as described in Helfgott et al. (1985) 162: 1531-1545.

Adjuvant arthritis and collagen arthritis were induced as follows. Adjuvant arthritis was produced by injecting rats intradermally on day 0 with 0.1 ml complete Freund's adjuvant (CFA), which consists of desiccated, heat-killed Mycobacterium tuberculosis strain H37Ra (Difco Laboratories, Detroit, MI) finely ground with a tissue pulverizer and added to incomplete Freund's adjuvant (IFA) at a concentration of 10 mg/ml oil (Helfgott et al. (1988) J. Immunol. 140;1838-1843). Collagen arthritis was induced by injecting rats intradermally with 0.4 mg of CII solubilized in 0.5 ml 0.1M acetic acid and emulsified in 0.5 ml IFA (Helfgott et al. (1985) J. Exp. Med. 162:1531-1545). Alternatively, collagent arthritis can be induced in mice by immunization with CII and CFA.

Therapeutic Use

Affinity purified AAF can be stored in a lyophilized state at -20°C . For treatments, AAF is solubilized in physiologic saline at a concentration of 100 $\mu\text{g}/\text{ml}$, sterilized by passage through 0.22 μ membrane filters (Millipore Corp., Bedford, MA), and kept in glass vials at 4°C . Endotoxin or animal virus contamination must be avoided as such contamination would result in toxic preparations.

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Other Embodiments

Other embodiments are within the following claims, for example, a DNA sequence encoding AAF can be isolated, cloned, and expressed in an expression system according to methods well known to those skilled in the art. Fragments of AAF, generated by methods well known to those skilled in the art, e.g., by proteolytic degradation or by genetic engineering, that possess the ability to reduce the incidence or severity, or to delay the onset of arthritis in rats immunized with collagen are also within the invention. Analogs of the AAF or analogs of fragments of AAF having minor variations from the native form, e.g., minor variations in amino acid sequence or in glycosylation, and the DNA sequences that encode these analogs, are within the claims if the analogs possess the ability to reduce the incidence or severity, or to delay the onset of arthritis in rats immunized with collagen. These fragments and analogs can be screened for activity as described above or by standard methods well known to those skilled in the art. AAF can be used as a vaccine to prevent or delay the onset of a collagen-autoimmune disease.

What is claimed is:

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1 1. A method for isolating a TABM capable of
2 suppressing an autoimmune response characteristic of a
3 human autoimmune disease, said method comprising
4 culturing T-cells which have been exposed to an
5 antigen to which patients with said disease produce an
6 autoimmune response, to stimulate TABM production by said
7 T-cells,

8 isolating from said culture cells, or their
9 supernatant, material capable of binding to said antigen
10 to produce an antigen-binding fraction, and

11 isolating from said antigen-binding fraction a
12 sub-fraction capable of delaying the onset or alleviating
13 symptoms of said autoimmune disease.

1 2. The method of claim 1 wherein said T-cells are
2 obtained from a human patient suffering from said
3 autoimmune disease.

1 3. The method of claim 1 wherein said T-cells are
2 obtained from a non-human mammal which has been immunized
3 with said antigen.

1 4. The method of claim 2 or claim 3, wherein said
2 T-cells are cultured in the presence of said antigen.

1 5. A substantially pure protein having the
2 following characteristics:

3 (a) it has a mass of approximately 55 kd as
4 determined by SDS-PAGE;

5 (b) it is found in collagen stimulated T-cell
6 antigen binding molecule secreting cells;

7 (c) it is capable of binding collagen;

8 (d) it reduces the incidence and severity and
9 delays the onset of arthritis in rats immunized with
10 collagen; and

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11 (e) it increases the titer of anti-collagen
12 antibodies when injected into collagen immunized rats.

1 6. The protein of claim 5, wherein the amino acid
2 composition is ASX 9.3; GLX 11.5; SER 13.8; GLY 10.4; HIS
3 1.3; ARG 3.1; THR 6.2; ALA 7.7; PRO 5.9; TYR 3.2; VAL
4 7.7.; MET 2.9; ILE 3.4; LEU 6.6; PHE 3.0; LYS 3.9.

1 7. The protein of claim 5 wherein the N-terminal
2 sequence is ASP-SER-LEU-THR-GLN-PRO-SER.

1 8. A DNA sequence encoding the protein of claim
2 5.

1 9. An expression vector containing the DNA
2 sequence of claim 8.

1 10. A cell transformed with the vector of claim
2 9.

1 11. A method of producing the protein of claim 5
2 comprising
3 culturing the cell of claim 10 and
4 isolating the protein from the cultured cell
5 or supernatant.

1 12. The protein of claim 5, in a formulation and
2 unit dosage sufficient to decrease the symptoms of an
3 auto-immune disease in a mammal.

1 13. The protein of claim 12, wherein said animal
2 is human.

1 14. The protein of claim 12, wherein said disease
2 is inflammatory arthritis.

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1 15. The protein of claim 12, wherein said disease
2 is rheumatoid arthritis.

1 16. The protein of claim 12, wherein said disease
2 is juvenile rheumatoid arthritis.

1 17. The protein of claim 12, wherein said disease
2 is psoriatic arthritis.

1 18. The protein of claim 12, wherein said disease
2 is autoimmune uveitis.

1 19. The protein of claim 12, wherein said disease
2 is polychondritis.

1 20. The protein of claim 12, wherein said disease
2 is idiopathic bilateral progressive sensorineural hearing
3 loss.

1 21. The fragment of the protein described in
2 claim 5, said fragment possessing the following
3 characteristics:

4 (a) it reduces the incidence and severity and
5 delays the onset of arthritis in rats immunized with
6 collagen; and

7 (b) it increases the titer of anti-collagen
8 antibodies when injected into collagen immunized rats.

1 22. A DNA sequence encoding the fragment of claim
2 21.

1 23. The expression vector containing the DNA
2 sequence of claim 22.

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1 24. A cell transformed with the vector of claim
2 23.

1 25. A method of producing the fragment of claim
2 21 comprising
3 culturing the cell of claim 24;
4 isolating the protein from the cultured cell
5 or supernatant.

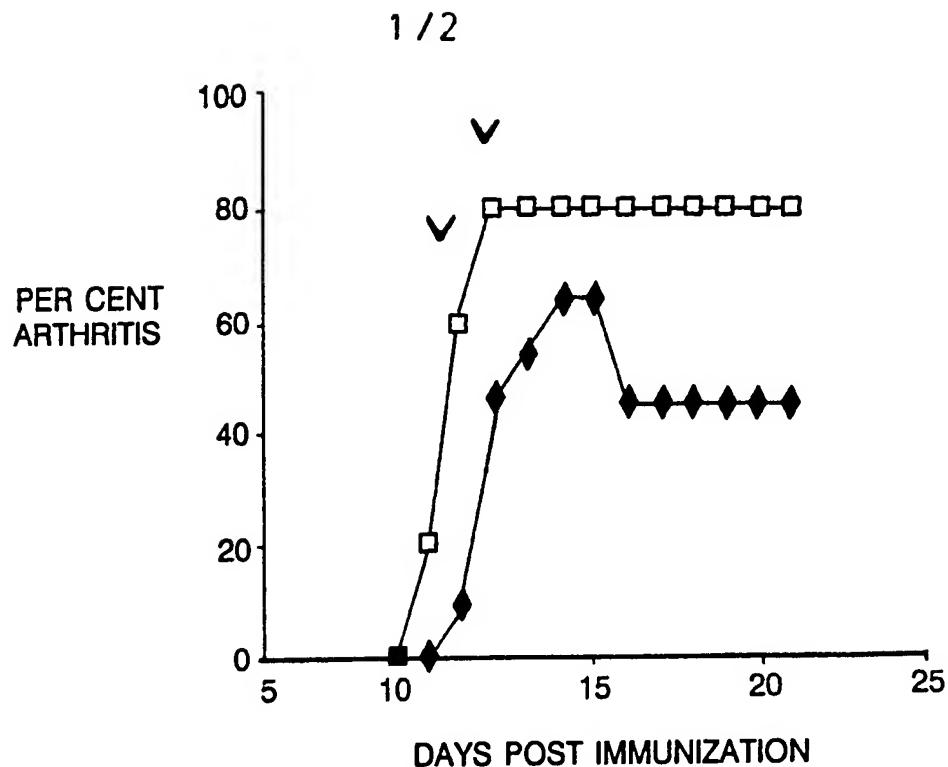
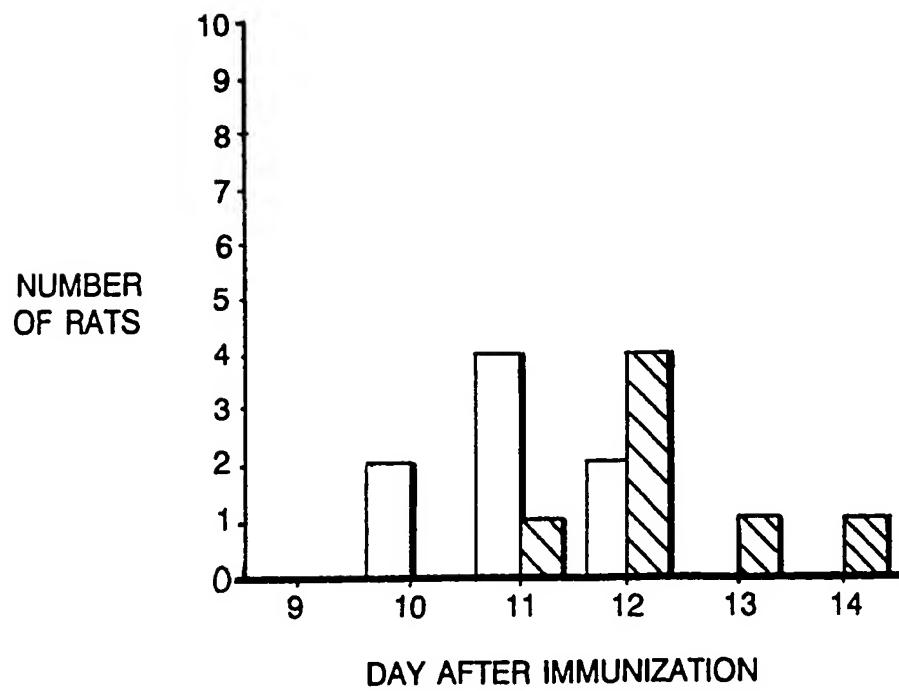


FIG. 1

FIG. 2
SUBSTITUTE SHEET

2 / 2

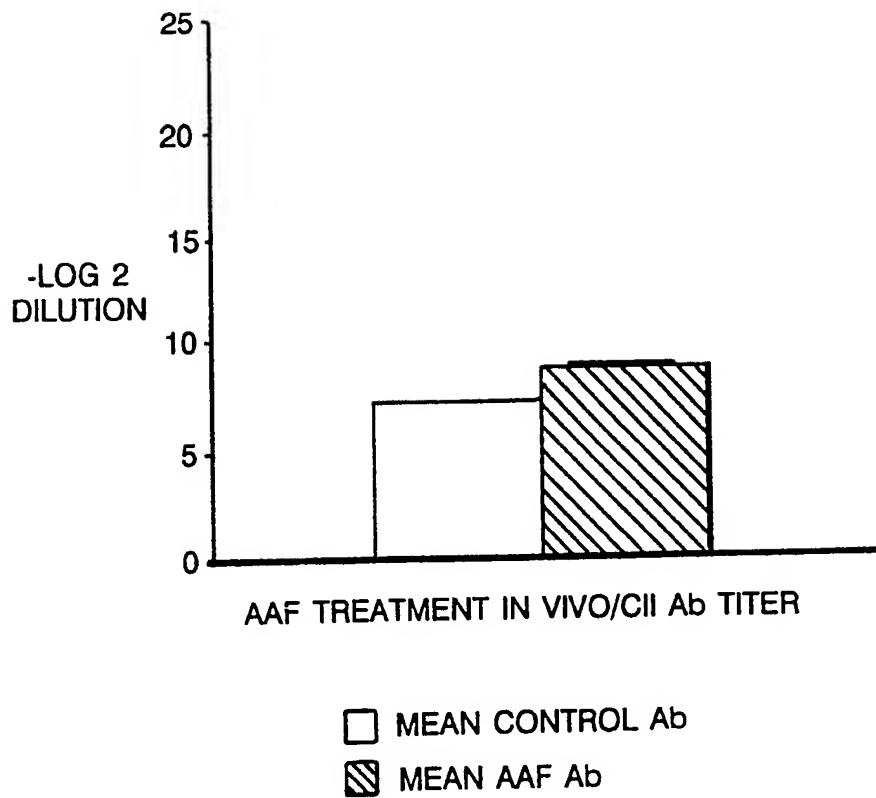


FIG. 3

SUBSTITUTE SHEET

Best Available Copy

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US91/02312

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, list them)

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): A61K 39/00, 45/05; C12N 1/00, 5/10

U.S. Cl: 435/69.1; 530/351; 536/27; 514/2, 15

II. FIELDS SEARCHED

Minimum Documentation Searched¹

Classification System	Classification Symbols
U.S.	435/69.1; 514/2, 15; 530/351; 536/27

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched²

Databases: Dialog (files 5,399,154, 73,155, 357), USPQ

Automated Patent System (file USPAT, 1971-1991)

III. DOCUMENTS CONSIDERED TO BE RELEVANT³

Category ⁴	Citation of Document, ⁵ with indication, where appropriate, of the relevant passages ⁶	Relevant to Claim No. ⁷
V	The Journal of Experimental Medicine, Vol. 154, issued 1981, Cremer et al., "Auricular chondritis in Rats An experimental model of relapsing polychondritis induced with type II collagen" pages 535-540, see entire document.	1-5 11-20
V	The Journal of Experimental Medicine, Vol. 162, issued November 1985, Helfgott et al., "An arthritogenic lymphokine in the rat," pages 1531-1545, see entire document.	1-5, 11-20
V	The Journal of Immunology, vol. 140, no. 6, issued 15 March 1985, Helfgott et al.	1-5, 11-20

* Special categories of cited documents:⁸

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document related or prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or practice underlying the invention

"C" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"R" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

5 Document number of the corresponding claim

IV. CERTIFICATION

Date of the Actual Completion of the International Search

Date of Mailing of the International Search Report

04 June 1991

25 JUN 1991

International Bureau - WIPO

TSA/IS

Lynette Smith
for
Jerome Morris

Best Available Copy

International Application No. PCT/US91/02312

PCT/US91/02312

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		D SHEET)
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	<p>Detection of Arthritogenic factor in Adjuvant Arthritis", pages 1838-1843, see entire document.</p> <p>US, A, 4,677,064 (Mark et al) 30 June 1987, see entire document.</p>	<p><u>6, 7</u> 3-10, 21-24</p>